AUGUST 1991

# ANIMAL HEALTH CONDITIONS FOR IMPORTATION INTO GREAT BRITAIN OF FROZEN OVINE EMBRYOS FROM USA (N.E. STATES ONLY)

The embryos shall not be landed in Great Britain unless and until there is delivered to an officer of Customs and Excise at the port/airport of landing a license issued under the Importation of Embryos, Ova and Semen Order 1980 and a certificate signed by a designated accredited veterinarian and endorsed by a full-time salaried veterinary officer of the Federal Government of USA stating:

## DETAILS

1. (i) the name and address of the approved embryo collection centre or farm at which the donor sheep were resident during the period of collection of embryos for export to Great Britain, together with the dates of residence on that centre or farm;

   (ii) the addresses, with dates of residence, of all premises where the donor sheep were resident during the 12 months prior to the entry into the approved embryo collection centre or farm;

   (iii) the breed, ear numbers (or other identification marks), of the donor sheep;

   (iv) EITHER (a) the identification of the donor ram from which the semen was collected, dates of collection, place of collection; only semen which has been collected in accordance with the conditions at paragraph 17B may be used;

   OR (b) the identification of the ram which was used for natural service in accordance with paragraph 17A;

   (v) the dates of collection of the embryos to be exported;

   (vi) the number of ampoules/straws;

   (vii) the number of embryos in the consignment;

   (viii) the indelible identification marking on the ampoules of embryos;

   (xi) and the individual identification marking on the official tamperproof seal of container/flask containing embryos for export.

## COUNTRY DISEASE CLEARANCE

2. No clinical or other evidence of contagious caprine pleuropneumonia, foot and mouth disease, lumpy skin disease, pestes des petits ruminants, rinderpest, Rift Valley fever, sheep pox or goat pox has occurred in the USA during the 12 month period prior to collection and until the date of despatch of the embryos to Great Britain. Vaccination against these diseases has not been practiced during the period.

## STATE DISEASE CLEARANCE

3. No clinical evidence of bluetongue, epizootic hemorrhagic disease of deer or vesicular stomatitis has occurred in any State where the donor animals have been resident during the 6 month period prior to the collection of embryos for export and until the date of despatch of the embryos to Great Britain.

## RESIDENCY OF DONOR SHEEP

4. (a) The donor sheep have been continually resident in the following States for at least 6 months prior to the collection of the embryos;

   Connecticut, Indiana, Maine, Massachusetts, Michigan, Minnesota, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, Vermont, Wisconsin, Delaware, Maryland, North Dakota, and West Virginia;

   (b) have had no contact with sheep other than those born in the USA or

imported direct from Great Britain within the previous 12 months;

(c) originate from premises where the flock has been in existence as an identifiable flock for a minimum period of 12 months.

PREMISES CLEARANCES

5. The flock(s) of origin of all donor females has/have been free of any evidence of brucellosis (including B. abortus, B. melitensis, and B. ovis) for the past 24 months.

6. (a) The donor animals originated from premises on which no clinical, serological or pathological evidence was found which leads to the conclusion that rabies, scrapie, maedi-visna/caprine arthritis encephalitis syndrome, listeriosis, leptospirosis, contagious agalactia (including Mycoplasma agalactia, M. arginine, M. bovis, M. capricolum, M. mycoides subspecies mycoides large colony type), Campylobacter fetus infection and caseous lymphadenitis (Corynebacterium ovis infection) has occurred during the 12 months prior to their movement to the embryo collection centre.

(b) The donor animals originated from premises on which there was no clinical or pathological evidence of ovine enzootic (chlamydial) abortion during the 12 months prior to their movement to the embryo collection centre.

TESTING OF FLOCK OF ORIGIN (MALE AND FEMALE)

7. (i) Within 12 months immediately prior to entry of the donor animals into the approved embryo collecting centre, all sheep over 12 months of age in the flock of origin including the donor females and the donor males when natural service is used, were subjected on two occasions to the agar-gel immunodiffusion test or enzyme linked immunosorbent assay for maedi/visna (M/V) (using the maedi/visna or CAE antigen) with negative results. The interval between the two tests shall be not less than 6 months, and the second test shall be carried out within the 6 months immediately prior to entry into the approved embryo collection centre.

(ii) Within the 6 months immediately prior to entry into the approved embryo collection centre, all rams in flocks with 20 or more breeding rams, or in flocks with fewer than 20 breeding rams, all rams and 5% of the breeding females to include the donor females (with a minimum of 20 females) were subjected to the complement fixation test for Brucella ovis (negative is a reaction of less than 50 i.u.s.);

(iii) Within the 6 months immediately prior to entry into the approved embryo collection centre, all sheep over the age of 12 months in the flock of origin and including the donor sheep were subjected to the following tests with negative results:

the complement fixation test for contagious agalactia using M. agalactia, M. bovis, M. capricolum and M. mycoides subspecies mycoides large colony antigens (negative is a reaction of less than 50% fixation at a dilution of 1 in 20, using the GB protocols supplied).

8. (i) Following the first flock test at paragraph 14 (i) and until the removal of the donor sheep to the embryo collection centre, no sheep were added to the flock except by direct movement form flocks which had undergone similar herd tests for maedi/visna, Brucella ovis and contagious agalactia (as in paragraphs 7 (i) (ii) and (iii) with negative results within 12 months immediately preceding their movement;

(ii) Any flock with which the flock of origin of the donor sheep has been in contact during the 12 months prior to export must comply with paragraphs 7(i) and (ii) and 8(i) above.

ON FARM ISOLATION AND TESTING OF DONOR SHEEP (LEPTOSPIROSIS OPTION ONLY)

9. For the purpose of exercising this treatment option only:-

EITHER (i) The donor sheep were isolated on their farm(s) of origin ("on-farm isolation") for at least 15 days immediately preceding movement to the approved embryo collection centre;

OR (ii) in the case of donor sheep moving to an embryo collection centre situated on the same farm premises, they were isolated for at least 15 days in the embryo collection centre.

10. During the period of isolation under this option the donor sheep were subjected to the following:

2 injections of dihydrostreptomycin (25 mg per kg live bodyweight) at an interval of 14 days, the second injection being made within 24 hours of the collection of embryos or of semen used to prepare embryos.

11. Insofar as it can be ascertained neither the donor animals nor their sires, dams, siblings and offsprings have shown any evidence of genetic defects.

MOVEMENT TO APPROVED EMBRYO COLLECTION CENTRE

12. The donor females and males, if applicable, were moved to the approved embryo collection centre in road/rail vehicles which had been thoroughly cleansed and disinfected and while in transit were not unloaded and did not come into contact with animals not similarly certified.

APPROVED EMBRYO COLLECTION CENTRE FOR EXPORT TO GB

13. The embryo collection centre or farm must be situated in one of the States listed at paragraph 4.

14. An embryo collection centre for the export of embryos to Great Britain must be approved for the purpose by a full-time salaried veterinary officer of the Government of the USA, using Great Britain protocols as attached at Appendix 1 or 2 (as appropriate).

15. The accredited veterinarian must satisfy himself that the collection, processing, packaging and freezing and transfer to quarantine of embryos for export to Great Britain were done in accordance with agreed protocols.

16. All embryos for export to Great Britain must be collected, processed, packaged and frozen at an approved embryo collection centre, or by the approved centre staff on the farm where the donor female is resident.

SEMEN USED

17. EITHER (A) Donor females have been served naturally by rams derived from the herds of origin which have been isolated and subjected to the same testing programme as the donor females, and have passed all tests with negative results;

OR (B) The semen used to inseminate donor females:

OR

(B) had complied in full with any current animal health conditions for the importation of frozen ovine semen into Great Britain from the USA.

EMBRYO COLLECTION AND PROCESSING

18. At the time of each collection, the donor females were clinically examined by an accredited veterinarian designated by the Federal Government of the USA, and showed no signs or symptoms of infectious or contagious disease.

19. The embryos were collected, handled and washed in accordance with the following

protocols and to the satisfaction of the accredited veterinarian:

(i) separate sterile equipment shall be used for the handling and washing of embryos from each donor female;

(ii) embryo washing and other "disinfection" treatment must precede freezing;

(iii) the zona pellucida of each embryo must be examined over its entire surface area at not less than 50x magnification and be intact and free from adherent material;

(iv) embryos must be washed by transferring them through ten changes of medium. Each wash must constitute a 100-fold dilution of the previous wash, and a new sterile pipette must be used to transfer the embryos on each occasion.

(v) Any product of animal origin used for the washing of embryos must be prepared in accordance with internationally agreed standards and include the addition of antibiotics. (OIE International Zoo-Sanitary Code 5th Edition Appendix 5.2.3.1.). If serum is used, it must be subjected to a temperature of 56°C for at least 1/2 hour prior to use.

20. The embryos were identified and sealed in ampoules/straws on which the date of collection of the embryos and other necessary identification marks were recorded indelibly.

21. All embryos for export to Great Britain must be processed and frozen in equipment which has been cleansed and disinfected prior to the processing of embryos for export to Great Britain.

EXAMINATION OF FLUSHING FLUID

22. At the time of each collection of the embryos for export, the total flushing fluid must be allowed to settle for 1 hour and be decanted, so that the final 50 mls of flushing fluid containing the embryos, can be retained. If the filtration method is used, the final 50 mls of flushing fluid containing the uterine debris must be retained.

After removal of the viable embryos, the 50 mls samples must be sent to the National Veterinary Services Laboratory, Ames, Iowa, for inoculation into two susceptible test sheep which have been subjected within the previous 28 days to the agar-gel immunodiffusion test or the blocking ELISA for bluetongue, the agar-gel immunodiffusion test for epizootic hemorrhagic disease virus, and the serum neutralization test for vesicular stomatitis with negative results. Samples may be pooled from up to 5 collections (including multiple donors) for inoculation into one test sheep but failure of the tests at paragraph 24 will result in the inadmissibility for export of the embryos from these animals.

23. The flushing fluid/medium for inoculation into sheep was handled in accordance with the protocols at Appendix 3.

TEST SHEEP FOR FLUSHING FLUID

24. After a period of at least 28 days from the date of inoculation, the test sheep was subjected to the following tests with negative results:

(i) the agar-gel immunodiffusion test or the blocking ELISA for bluetongue;

(ii) the agar-gel immunodiffusion test for epizootic hemorrhagic disease virus using Alberta and New Jersey antigens;

(iii) the serum neutralization test for vesicular stomatitis, using New Jersey and Indiana antigens;

POST COLLECTION TESTING OF DONOR FEMALES

25. After a period of at lease 28 days has elapsed from the date of the last collection of embryos for export, the donor females were subjected to the following with negative results:

(i) the complement fixation test for brucellosis using Brucella abortus antigen (negative is a reaction of less than 10 icftu/ml);

(ii) the complement fixation test for brucellosis using Brucella ovis antigen (negative is a reaction of less than 50 i.u.);

(iii) the agar-gel immunodiffusion test or enzyme linked immunosorbent assay for maedi/visna (M/V) (using the maedi/visna or CAE antigen);

(iv) the complement fixation test for mycoplasmosis using Mycoplasma agalactia, M. arginine, M. bovis, M. capricolum and M. mycoides subspecies mycoides large colony antigens (negative is less than 50% fixation at the dilution of 1 in 20);

(v) the agar-gel immunodiffusion test or the blocking enzyme linked immunosorbent assay (ELISA) for bluetongue;

(vi) the agar-gel immunodiffusion test for epizootic hemorrhagic disease of deer virus using Alberta and New Jersey antigens.

AND if the treatment option is not used at paragraph 10:-

(vii) the microscopic agglutination test using live antigen for leptospira, serotypes australis, bratislava, grippotyphosa, hardjo, pomona and tarassovi (negative is less than 50% agglutination at a serum dilution of 1 in 100);

EMBRYO STORAGE AND QUARANTINE

26. The ampoules/straws containing the embryos for export to Great Britain were stored in separate flasks which had been emptied and thoroughly cleansed and disinfected since any previous use and contain fresh liquid nitrogen not previously used for any other purpose.

27. After processing and until dispatch to Great Britain (shich shall not be until the results of any tests at paragraph 25 are available, and an import license issued), the embryos for export to Great Britain must be stored under quarantine conditions approved by the Federal Government of the USA and under the control of a veterinarian designated by the Federal Government of the USA.

SHIPMENT OF EMBRYOS

28. The flask in which the embryos are being consigned to Great Britain was sealed immediately prior to export with an official tamperproof seal in the presence of a full-time salaried veterinary officer of the Federal Government of the USA.

NOTES

1. The dates of all examinations, tests and treatments (as applicable) required by the protocol must be declared within the export health certification.

2. All laboratory tests required by this protocol must be carried out at the National Veterinary Services Laboratory (NVSL), Ames, Iowa.

3. Applications for import licenses must be submitted to the Ministry/Department as under:

For port of entry in England - The Secretary, Animal (International Trade) Health Division, Ministry of Agriculture, Fisheries and Food, Hook Rise South, Tolworth, Surbiton, Surrey KT6 7NF.

For port of entry in Scotland - Scottish Office Agriculture and Fisheries Department, Pentland House, 47 Robbs Loan, Edinburgh.

For port of entry in Wales - Welsh Office Agriculture Department, Cathays Park, Cardiff, Wales CF1 3NQ.

4. At the time of application for an import license the original animal health

certificate must be submitted for scrutiny. Paragraphs relating to country disease clearance and sealing of flasks need not be completed at this time. This certificate will be returned and must accompany the embryos into Great Britain after it has been finally completed and signed by the certifying veterinary officer.

APPENDIX 1 AUGUST 1991

## PROTOCOLS FOR THE APPROVAL OF A PERMANENT EMBRYO COLLECTION CENTRE FOR EXPORT OF EMBRYOS TO GREAT BRITAIN FROM THE UNITED STATES

### APPROVAL

1. The embryo collection centre and the quarantine accommodation should be approved by a full-time salaried veterinary officer of the Federal Government of the USA.

### STRUCTURE

2. A permanent embryo collection centre must be premises specifically built or adapted for the purpose within a perimeter fence to which animals are consigned as donors. Livestock within the centre must have no contact with other livestock.

3. There should be adequate housing and yards for the maximum number of stock likely to be resident at any one time.

4. Accommodation should be provided for each of the following purposes:

   (i) housing of donors;
   (ii) preparation of donors;
   (iii) collection of embryos;
   (iv) recovery room, if surgical collections are carried out;
   (v) laboratory for recovery and processing of embryos (see paragraph 19);
   (vi) adequate changing rooms, washing and disinfection facilities and toilets.

5. The accommodation at paragraph 4 which is used for the donor animals, and for the collection, processing, freezing and storage of embryos must be constructed and maintained so that all surfaces can be effectively cleansed and disinfected.

6. Natural and artificial drainage should be such that the risk of disease to the centre is minimal.

### MANAGEMENT

7. A register must be kept of all animals at the centre giving details of breed, place of birth, date of entry to the centre, full testing and health history and identification of each of the animals.

8. The premises housing the donor females shall be maintained in a sanitary manner to the satisfaction of the accredited veterinarian designated by the Federal Government.

9. From the time of disinfection of the approved premises and during the whole of the collection period there must be no livestock other than the donor females on the collection centre.

10. Arrangements for the collection of milk and delivery of feedstuff and other goods should be such as to ensure that drivers and their vehicles do not come into contact with livestock on the collection centre.

11. Authorized visitors may be permitted according to conditions laid down by the accredited veterinarian.

12. The centre must employ staff suitably trained in techniques relevant to the control of the spread of disease.

### SUPERVISION

13. The centre must be under the supervision of an accredited veterinarian.

14. Staff working at the centre should be provided with separate protective clothing for use on the centre. Effective personal disinfection procedures shall be carried out by all personnel entering and leaving the centre.

15. The collection centre and quarantine accommodation must be supervised so that the collection, processing, storage and quarantine of embryos takes place only in accommodation set aside for the purpose under conditions of strictest hygiene.

16. The embryos for export to Great Britain must be processed, frozen and stored at the beginning of each day before any embryos, which are not for export, are handled at the approved centre.

17. All equipment having contact with embryos or donor animals during collection must be appropriately disinfected and sterilized prior to use.

RECORDS

18. Detailed records including dates should be kept on the collection centre of:-

(i) collections carried out including identification of donors;
(ii) the culture and preservative media used;
(iii) number of embryos collected;
(iv) identification of the sire and dam of every embryo;
(v) transfer of embryos into storage.

PROCESSING EMBRYOS

19. A suitable laboratory should be provided. A mobile laboratory and equipment preparation vehicle may be used but this should be cleansed and disinfected under the supervision of an accredited veterinarian before entry into the collection unit.

20. All equipment should either be disposable and used once, or cleansed and sterilized before reuse. Liquid nitrogen containers must be capable of being cleansed and disinfected.

21. Freezing equipment must be cleansed by appropriate means before use for the freezing of embryos for export to Great Britain. No other embryos should be frozen at the same time.

22. After freezing, embryos for export to Great Britain shall be transferred to the embryo quarantine accommodation which must be kept locked.

STORAGE OF EMBRYOS

23. The embryos for export to Great Britain must be stored in separate flasks which had been emptied and thoroughly cleansed and disinfected since any previous use and contained liquid nitrogen not previously used for any other purpose.

24. After processing and until despatch to Great Britain the embryos for export to Great Britain must be stored in the approved quarantine accommodation, under quarantine conditions controlled by an accredited veterinarian.

APPENDIX 2 AUGUST 1991

## PROTOCOLS FOR THE APPROVAL OF AN ON FARM EMBRYO COLLECTION CENTRE FOR EXPORT OF EMBRYOS TO GREAT BRITAIN FROM THE UNITED STATES

APPROVAL

1. The embryo collection centre and the quarantine accommodation should be approved by a full-time salaried veterinary officer of the Federal Government of the USA.

STRUCTURE

2. An on farm collection centre will be constructed or adapted for the purpose by a farmer on his own premises. The embryo collection centre must be so situated

that livestock within the centre has no contact with animals from other premises.

3. There should be adequate housing and yards for the maximum number of stock likely to be resident at any one time.

4. Accommodation should be provided for each of the following purposes:

(i) housing of donors;
(ii) collection of embryos;
(iii) laboratory for the recovery and processing of embryos (see paragraph 19).

5. The accommodation at paragraph 4 must be constructed and maintained so that all surfaces can be effectively cleansed and disinfected.

6. Natural and artificial drainage should be such that the risk of disease to the centre is minimal.

MANAGEMENT

7. A register must be kept of all animals at the centre giving details of breed, place of birth, date of entry to the centre, full testing and health history and identification of each of the animals.

8. The premises housing the donor females shall be maintained in a sanitary manner to the satisfaction of the accredited veterinarian designated by the Federal Government.

9. From the time of disinfection of the approved premises and during the whole of the collection period there must be no livestock other than the donor females on the collection centre.

10. Arrangements for the collection of milk and delivery of feedstuff and other goods should be such as to ensure that drivers and their vehicles do not come into contact with livestock on the collection centre.

11. Authorized visitors may be permitted according to conditions laid down by the accredited veterinarian.

12. The centre must employ staff suitably trained in techniques relevant to the control of the spread of disease.

SUPERVISION

13. The centre must be under the supervision of an accredited veterinarian.

14. Staff working on the centre should be provided with separate protective clothing for use on the centre. Effective personal disinfection procedures shall be carried out by all personnel entering and leaving the centre.

15. The collection centre and quarantine accommodation must be supervised so that the collection, processing, storage and quarantine of embryos takes place only in accommodation set aside for the purpose under conditions of strictest hygiene.

16. The embryos for export to Great Britain must be processed frozen and stored at the beginning of each day before any embryos which are not for export, are handled at the approved centre.

17. All equipment having contact with embryos or donor animals during collection must be appropriately disinfected and sterilized prior to use.

RECORDS

18. Detailed records including dates should be kept on the collection centre of:-

(i) collections carried out including identification of donors;
(ii) the culture and preservative media used;
(iii) number of embryos collected;
(iv) identification of the sire and dam of every embryo;
(v) transfer of embryos into storage.

PROCESSING EMBRYOS

19. A suitable laboratory should be provided. A mobile laboratory and equipment preparation vehicle may be used but this should be cleansed and disinfected under the supervision of an accredited veterinarian before entry into the collection unit.

20. All equipment should either be disposable and used once, or cleansed and sterilized before reuse. Liquid nitrogen containers must be capable of being cleansed and disinfected.

21. Freezing equipment must e cleansed by appropriate means before use for the freezing of embryos for export to Great Britain. No other embryos should be frozen at the same time.

22. After freezing, embryos for export to Great Britain shall be transferred to the embryo quarantine accommodation which must be kept locked.

STORAGE OF EMBRYOS

23. The embryos for export to Great Britain must be stored in separate flasks which had been emptied and thoroughly cleansed and disinfected since any previous use and contained liquid nitrogen not previously used for any other purpose.

24. After processing and until despatch to Great Britain the embryos for export to Great Britain must be stored in the approved quarantine accommodation, under quarantine conditions controlled by an accredited veterinarian.

APPENDIX 3 AUGUST 1991

IMPORTATION INTO GREAT BRITAIN OF FROZEN OVINE EMBRYOS
FROM THE UNITED STATES

PROTOCOLS FOR EXAMINING EMBRYOS FLUSHING FLUID/MEDIUM FOR BLUETONGUE, EHDV AND VESICULAR STOMATITIS

1. At the time of each collection of embryos for export and under the supervision of an accredited veterinarian the final 50mls of flushing fluid must be retained from each potential donor after removal of the embryos.

2. The flushing fluid should be collected according to normal veterinary practice intended to yield a sample with minimum contamination.

STORAGE

3. The flushing fluid must be placed in a sterile container and held at +4°C packed in ice to assist cooling.

4. The flushing fluid must be transported in insulated containers at +4°C to the National Veterinary Services Laboratory, Ames, Iowa, for testing within 24 hours of collection. If this is not possible the samples should be stored at -70°C until they can be tested.

5. After arrival at the National Veterinary Services Laboratory, Ames, Iowa, the flushing fluid should be held at +4°C and should either be inoculated into sheep on the day it arrives at Ames or be divided into two volumes as described at paragraph 6a frozen and stored at -70°C.

METHODOLOGY

6. Divide the flushing fluid into two equal volumes and inoculate a minimum of 20 ml by the intra-peritoneal route into one bluetongue and EHDV susceptible sheep. The remainder is stored at (or remains stored at) -70°C for emergency use. Samples may be pooled from different donors or collections but failure of any of the tests will result in the inadmissibility for export of all embryos from these animals. Flushing fluids from a maximum of 5 collections may be pooled and inoculated into one sheep.

SHEEP

7. The susceptible sheep used for each test must have been subjected to the agar-gel immunodiffusion test or the blocking ELISA for bluetongue, the agar-gel immunodiffusion test for epizootic hemorrhagic disease virus and the serum neutralization test for vesicular stomatitis using New Jersey and Indiana antigens with negative results within 28 days prior to the date of inoculation.

8. The test sheep must be maintained in locked, high security accommodation which should be approved by and under the direct supervision of a duly authorized officer of the Government of the USA.

9. The test sheep must be examined daily for clinical signs of disease for 28 days following inoculation. A full investigation must be made of any sheep found to be ailing.

SAMPLING AND TESTING OF SHEEP

10. After a period of at least 28 days from the date of inoculation the test sheep must be subject to the following tests with negative results.

    (i) the agar-gel immunodiffusion test or the blocking ELISA for bluetongue;

    (ii) the agar-gel immunodiffusion test for epizootic hemorrhagic disease virus using Alberta and New Jersey antigens;

    (iii) the serum neutralization test for vesicular stomatitis using New Jersey and Indiana antigens.

(The blocking ELISA shall be performed in accordance with Anderson, J. (1984) J. Immunol. Methods 74, 139-149.)

11. The sera collected from the test sheep prior to inoculation and at 28 days after inoculation with flushing fluid must be tested at the National Veterinary Services Laboratory, Ames, Iowa.